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SCIENTIFIC RESEARCH ON PAPAVER SPECIES AS SOURCES OF CODEINE, MORPHINE AND THEBAINE 1/

No. 24

Research in Norway on Papaver species designed to improve the yield of phenanthrene alkaloids and to produce quality seeds

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1/ This ST/SOA/SER.J/.. series of documents was previously entitled "Scientific research on Papaver bracteatum".

General note: The views expressed by collaborating scientists in their contributions to the programme are not necessarily shared by the Secretariat.

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INTRODUCTION

The following species and varieties of Papaver were used in the research programme: Papaver bracteatum (Arya I); Papaver bracteatum (Arya II); Papaver somniferum (12 varieties); Papaver orientale and Papaver pseudo-orientale.

Test cultivation of Papaver bracteatum (Arya I) was started in 1968. Plant improvement experiments (γ-radiation of seeds before planting and polyploidation experiments) were carried out over the last 3-4 years. Precursor incorporation experiments, with labelled and non-labelled precursors, were started in 1975 and went on in 1976. Analyses of seeds from Papaver bracteatum (Arya I), and analyses of the alkaloid content of several samples of the test cultivated species, have been carried out, or are in progress. Investigations on the low-molecular carbohydrate fraction, the higher carbon sugars and the water-soluble polysaccharides of Papaver somniferum have been performed and published. Comparative investigations on the organic acids in the above mentioned species of Papaver are in progress, and studies on the influence of ecological factors on the alkaloid content of several Papaver species was started last summer.

Appendix I shows maps of the experimental plots during the summer 1976.

Reports on work and results obtained prior to the 1976 season were given at the Bracteatum symposia in Geneva, December 1972; in Teheran, September 1973; in Beltsville, October 1974 and in Rennes and Paris, May 1976. Appendix II includes a list of publications on opium and Papaver species by A. Nordal and coworkers.

EXPERIMENTAL RESULTS

In the following a very brief review is presented on the research by our group on Papaver bracteatum and Papaver somniferum, to improve the yield of codeine, morphine and thebaine.

As is known, investigations of this kind involve months of field work, followed by harvesting and preservation of hundreds of samples, before the analyses can be completed and the value of the experiments determined.

We are now at the stage where chemical and radio-chemical analyses of our samples from the 1976 harvests are in progress, and I estimate that it will take at least two months to complete the analytical part of the programme.

1. Papaver bracteatum (Arya I)

As mentioned above test cultivation of this species was started in 1968. The main problem at that time was to find out if the plant could be cultivated in Norway. We know today this is quite possible if certain precautions are taken to insure that the roots of the plants survive during the winter season.

γ-Radiation experiments

The plants used for this investigation were raised from seed type UNB-2, 1972. (The same type of seeds was used also for the polyploidation experiments).

The radiated seeds were supplied by Dr. O.J. Braenden, U.N. Narcotics Laboratory, Geneva, in 1972. We used four samples that had been given 20, 40, 60 and 80 Rad respectively. Seeds germinated from the two lower levels of radiated samples only.

About 70% of the plants which grew died following transplanting or during the first and second winter in the field. The radiated seeds gave plants which, as a rule, were less vigorous than plants from non-radiated seeds (control plants) and only a few of them gave flowers and fruits in 1974, the second year after planting. During the winter 1975-76, there was much frost without snow cover, in the Oslo area and about one half of the remaining plants died. Of the plants which survived (about 30 all together) approximately 50% flowered and gave ripe fruits in 1976. They were collected and will be analysed for their thebaine content and for certain other constituents to determine to what degree they differ from the control plants.

Polyploidation experiments

Seeds from P. bracteatum (UNB-2, 1972) were treated with varying concentrations of colchicine before and during germination to obtain plants with a higher chromosome number than the parent plants. The experiments which were started in 1972 are still underway. Analyses have shown that some polyploidation has taken place, but the analytical part is not yet completed.

Chemical composition of the seeds

Table I shows comparative analyses of the content of lipids, proteins and carbohydrates in the original seed type UNB-2, 1972 and in the corresponding seeds derived from plants raised in Norway.

Table I

Type of seeds	Fat (ether extract) %	Proteins %	Carbohydrates %
Seeds of Papaver bracteatum UNB-2, 1972 from Iran	27,0	27,3	40,4
Seeds of Papaver bracteatum UNB-2, 1972 raised in Norway	18,4	26,0	49,9

The rather low content of fat and the rather high content of carbohydrates in the seeds raised in Norway, indicate that the Norwegian seeds were not quite ripe when analysed, but germination tests have shown that fertile seeds of P. bracteatum can be produced in Norway.

2. Papaver bracteatum, UNB-17 (Arya II)

The first seed sample of this selection was received from the U.N. Narcotics Laboratory in December 1973. The plants in Plot C (see Appendix I) were raised from these seeds.

In 1975 plants from Plot C were used for precursor incorporation experiments where labelled compounds of L-phenylalanine, L-tyrosine, L-glycine and urea were applied as precursors. A preliminary report on this investigation is given in UN Secretariat publication ST/SOA/SER.J/22, 30 April 1976. A paper on the same subject has been accepted for publication in Acta pharmaceutica Suecica.

Analyses carried out on capsules from Plot C (Arya II), 1975 crop, showed a thebaine content of 4.0%. Corresponding analyses of capsules from Plot A (Arya I, 1975) showed a thebaine content of 1.34%.

The successful experiments with labelled precursors in 1975 were followed, in 1976, with non-labelled precursors in order to find out if the expected raise in the relevant alkaloids content would be of such a magnitude that it could be determined by means of conventional chemical methods.

In 1976, Plot C (Arya II) was divided into four equal sections and the plants were sprayed once a week from June 3 to August 2, 1976 with the precursor solutions shown in Figure 1. On August 9 the plants were harvested and air dried. Analyses of the plant material are in progress.

3. Papaver somniferum

1. Plot B (see Appendix I) was sown in 1976 for two reasons:

(a) to produce seeds of Norwegian origin for ecological investigations;

(b) to perform precursor incorporation experiments with labelled and non-labelled precursors with this species in the same way as with Papaver bracteatum (Arya II).

The seeds used for this plot were of Turkish origin, and a good yield of fertile seeds (about 1 kg) was obtained.

Precursor incorporation experiments with labelled compounds (urea, phenylalanine and glycerine) and with a non-labelled compound (urea) were performed in the same way as described for Arya II, and the analyses of the plant material are in progress.

2. Plot H and Plot I (see Appendix I)

In both plots the same 12 varieties of Papaver somniferum were grown. Plot H was located in the Oslo area and Plot I north of the Arctic circle.

The reason why these plots were started in 1976 was to perform some pilot experiments in connexion with the above mentioned ecological investigations. Plot H was also expected to give more varieties of seeds for the same investigations.

The plants from the two plots were harvested at the end of September 1976 and dried. The seeds from the different varieties will be taken care of, and the aerial parts of the plants will be subjected to comparative analyses.

RESULTS AND DISCUSSION

During the last five years a good deal of work has been carried out in Norway on Papaver bracteatum and other Papaver species. The main scope of this work has been, in close collaboration with United Nations Narcotics Laboratory, to perform scientific investigations to improve the yield of phenanthrene alkaloids in certain poppy species.

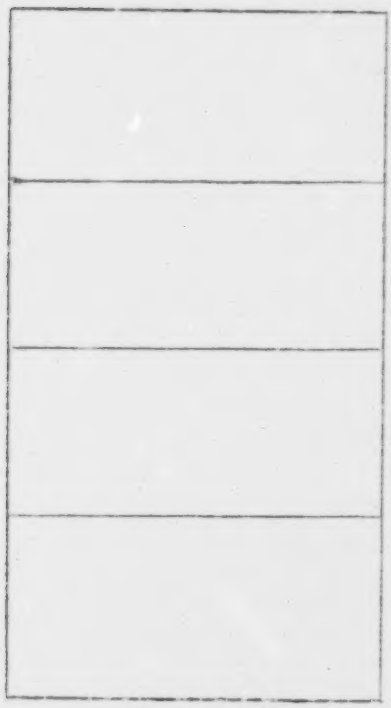
The investigations required a considerable amount of living plants and fresh and preserved material, which gradually was provided by cultivation of the relevant species at different places in Norway.

This first important, but time consuming, phase of the programme is now over, and the results are starting to accrue. For the time being two scientists from our staff, J.K. Wold, Ph.D., and B. Smestad Paulsen, Ph.D., plus a technician and myself are involved in the project.

The most promising results so far were the precursor incorporation experiments mentioned above. Also our chemical research might be important in elucidating the metabolic pathways, which in live plants lead to the production of the useful alkaloids (see the reference list in Appendix II).

Figure 1

Plot C (Arya II) 1976



Section 1: Control plants
(urea 1% in water)

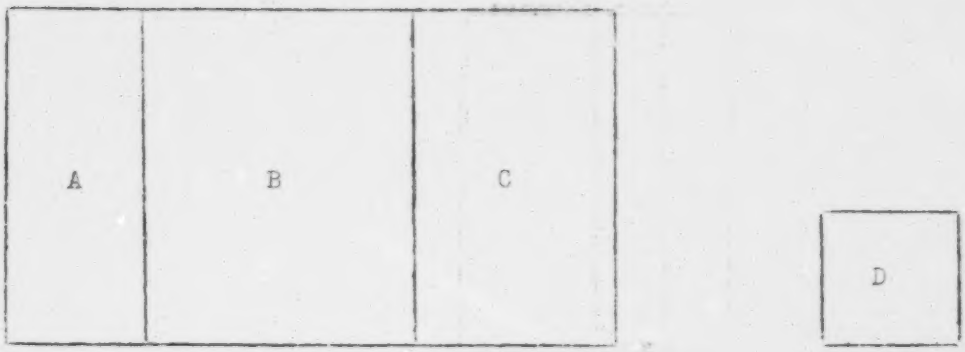
Section 2: Urea 1% in 1.0%
dimethylsulfoxide (DMSO)

Section 3: Urine : H_2O (1:1) in 0.1%
MON 0011

Section 4: Urea 1% in 0.1%
MON 0011

APPENDIX I

Research plots for Papaver species at Botanical Garden,
Tøyen, Oslo, Norway 1976



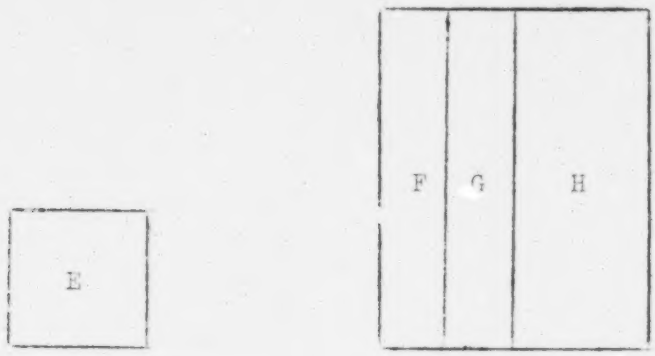
Plot A (100 m^2) : Papaver bracteatum (Arya I). Started 1968 for test cultivation, combined with chemical analyses of seeds, alkaloid content and organic acids.

Plot B (200 m^2) : Papaver somniferum. Started 1976. Used for labelled and non-labelled precursor incorporation experiments, for chemical analyses of alkaloids, organic acids and carbohydrates, and for seed production (see Plot H).

Plot C (150 m^2) : Papaver bracteatum (Arya II). Started 1974. Used for labelled and non-labelled precursor incorporation experiments and for chemical investigations.

Plot D (15 m^2) : Papaver bracteatum (Arya I). Started 1971 with γ -radiated seeds.

Research plots for Papaver species at the Phytotron area,
Blindern, Oslo, Norway 1976



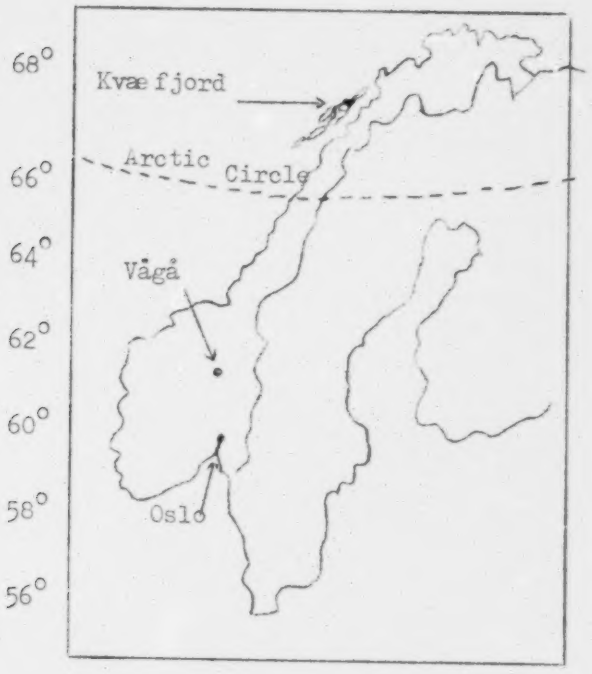
Plot E (15 m^2) : Papaver bracteatum (Arya I). Started 1972 for polyploidation experiments.

Plot F (50 m^2) : Papaver orientale. Started 1973, and used for comparative purpose (to study similarities and differences in the alkaloid and organi acid content of this species and the closely related Papaver bracteatum).

Plot G (50 m^2) : Papaver pseudo-orientale. Started 1973 and used in the same way as Plot F.

Plot H (100 m^2) : Papaver somniferum (12 different varieties). Started 1976. Intended for comparative analyses of alkaloid content (see Plot I, Kvæfjord), and for supply of seeds for ecological investigations.

Research plot for Papaver species at Kvaefjord, north of the Arctic Circle



Map of Norway, showing the location of Plot I, at Kvæfjord, north of the Arctic Circle.

Plot I (100 m^2) : Papaver somniferum (the same 12 varieties which are mentioned under Plot H).
Small "pilot" experiment to find out if it might be worthwhile to include this locality in the planned ecological programme of 1977.
(see Plot H).

APPENDIX II

List of publications and reports on opium and Papaver species by Arnold Nordal and coworkers

1. Nordal, A.: Natural and synthetic drugs with morphine-like effects evaluated from a pharmacognostic point of view. Bull. on Narcotics, VIII, No.1 (1956).

2. Nordal, A.: Final report covering study and possible exploitation of pharmaceutical raw materials available in Burma. Prepared for the Government of the Union of Burma, Rangoon (1959).

3. Nordal, A., E. Ottestad, E. Brochmannn-Hanssen and Dagrun Øiseth: The water-soluble carbohydrates of Papaver somniferum L. J. Pharmacy and Pharmacology, 11, 689 (1959).

4. Nordal, A. and M. Blyberg Falck: The assay, characteristics, composition and origin of opium. United Nations document ST/SOA/SER.K/131, 4 October 1969.

5. Nordal, A.: The medicinal plants and crude drugs of Burma. Medd. Norsk Farm. Selsk. 25, 127 (1963).

6. Nordal, A. and M. Blyberg Falck: Opium with 25 per cent morphine content produced in Norway. Norges Apot. for. Tidsskr., 72, 173 (1964).

7. Wold, J.K., B. Smestad, R. Winsnes and D. Resser: The water-soluble polysaccharide of opium poppy (Papaver somniferum L.). Acta Chem. Scand., 24, 1262 (1970).

8. Wold, J.K. and B. Smestad: The water-soluble polysaccharide of opium poppy (Papaver somniferum L.). Identification of acidic oligosaccharides from a partial acid hydrolysate. Acta Chem. Scand., 24, 2472 (1970).

9. Haustveit, G. and J.K. Wold: Higher-carbon sugars of opium poppy (Papaver somniferum L.). Acta Chem. Scand., 24, 3059 (1970).

10. Haustveit, G. and J.K. Wold: The low-molecular carbohydrate fraction of opium poppy (Papaver somniferum L.). Medd. Norsk Farm. Selsk., 31, 157 (1970).

11. Nordal, A.: Papaver bracteatum Lindl. Report on test cultivation and (plant) improvement experiments carried out in Norway during the years 1968-1972. First Working Group on Papaver bracteatum, Geneva, 12 December 1972.

12. Nordal, A.: A corresponding report at the Second Working Group on Papaver bracteatum, Teheran, 3 September 1973.

13. Nordal, A.: A corresponding report at the Third Working Group on Papaver bracteatum, Beltsville, Maryland, U.S.A., 7 October 1974.

14. Nordal, A.: Papaver bracteatum Lindl. Report on test cultivation, plant improvement experiments and feeding with alkaloid precursors. Fourth Working Group on Papaver bracteatum, Rennes and Paris, 16-23 May 1976.

15. Nordal, A. and J. Kvalevåg: En undersøkelse av alkaloidene i de norske fjellvalmue-artene Papaver lapponicum (A. Tolm) Nordh., Papaver laestadianum Nordh., Papaver dahlianum Nordh. og Papaver dubium L. Hovedfagsoppgave utført ved Universitetets Farmasøytiske institutt, Avd. for farmakognosi. Oslo, May 1974.

16. Nordal, A., B. Smestad Paulsen and J.K. Wold: Scientific Research on Papaver bracteatum. Precursor incorporation experiments in Papaver alkaloid biosynthesis. I. United Nations document ST/SOA/SER.J/22, April 1976.